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Multiplexed detection of molecular biomarkers with phase-change nanoparticles

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Abstract

This review describes a new biosensing method based on nanoparticles of solid-to-liquid phase-change materials, in which a panel of metallic nanoparticles (metals and eutectic alloys) that have different compositions and melting temperatures are used as thermal reporters. Each type of nanoparticle will be conjugated to a ligand that can specifically bind to one type of molecular biomarker (protein or DNA) and then immobilized onto a substrate that is comodified with multiple ligands by forming sandwiched antibody–antigen complexes or DNA double helices. After removing unbound nanoparticles by washing, the nature and concentration of the biomarkers are determined by detecting the melting temperature and fusion enthalpy of the nanoparticles using differential scanning calorimetry. Furthermore, an even larger panel of thermal barcodes can be formed by encapsulating selected phase-change nanoparticles inside non-melting shells, such as silica, where each microparticle will have a characteristic signature that can be determined from its thermal signatures.

Keywords

biomarker; detection; multiple; phase-change nanoparticle; thermal barcode

Need for multiplexed biomarker detection

A challenge of translating abundant molecular biology knowledge gained in the past into advances in disease detection to benefit patients is that most available molecular biomarkers are not sufficiently powerful, lacking either sensitivity or specificity, especially at early stages of diseases such as cancer. Take prostate-specific antigen (PSA) screening as an example [1], the biomarker has been widely used for detection and monitoring of prostate cancer, but increasing evidence indicates that PSA screening is not in itself adequate for the early detection of disease because it cannot distinguish lethal prostate cancers from indolent ones [2]. For patients who have 4–10 ng/ml PSA in the blood, the PSA test is not informative and, thus, a painful biopsy will be required to either confirm or rule out cancer. It is estimated that the widespread use of PSA in screening has led to overdiagnosis and

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over-treatment of approximately 1 million men in the USA with no clear mortality benefit [3], while other patients die from aggressive cancers that are diagnosed too late. Such a lack of specificity is because PSA can be produced at high abundance by normal prostate epithelial cells in transient or chronic inflammatory conditions, as well as benign prostatic hyperplasia. It has been found that having a PSA screen after cycling may lead to a higher PSA value [4]. The low power issue of single biomarkers is not limited to prostate cancers, but also found for other forms of cancers and other diseases, such as Alzheimer's disease and autism [5].

Existing techniques for biomarker detection

Parallel to efforts for finding more powerful or specific biomarkers [6], one feasible way of providing better predictive value is to detect multiple low-specificity biomarkers and analyze collective responses of multiple biomarkers contained inside one sample [7]. It is believed that such multiplexed detection will be able to enhance detection reliability and reduce the number of patients who must have invasive and painful biopsies [8,9]. However, the concentrations of biomarkers at the early stage of disease may span eight orders of magnitude, and some of them may have very low concentrations. It is challenging for current techniques to detect multiple biomarkers over a large range of concentrations from a small amount of sample [10]. Biomarkers may also be lost or irreversibly adsorbed on container walls during sample preparation. Although microarray is powerful in identifying biomarkers from specimens of later stages of disease [11], it is not suited for the detection of multiple biomarkers, whose concentrations span several orders of magnitude due to the heterogeneity of biomarkers at various stages of disease, identical feature size and capacity within an array to bind biomarkers [12]. The biomarker concentration may be too low to be detected or too high so as to cause signal saturation. In this aspect, systematic sample dilution or enrichment does not help, since the concentrations of each species will be changed at the same ratio. In addition, microarray cannot be used to detect proteins due to difficulties in pattern generation [13]. ELISA has high sensitivity [14], but is limited by signal saturation at high enzyme concentrations or after long incubations. PCR can generate errors due to nondiscriminative amplification of all DNA fragments. Although multiple protein and genetic biomarkers exist in samples, DNA microarray, PCR and ELISA only detect genetic or protein biomarkers, and cannot detect both protein and genetic biomarkers simultaneously.

Biomarker detection with nanoparticle probes

A variety of nanostructured materials with unique physical properties, including optical, electric, magnetic and electrochemical characters, can be used to detect cancer biomarkers owing to their ability to make intimate contact with biomolecules [15–18]. Nanoparticle-based detection of biomarkers can achieve extremely high sensitivities at the pico- or femto-mole/l level by converting biological recognition into measurable physical signals that can be amplified [19–21]. However, the multiplicity of existing nanoparticle detections is intrinsically limited due to their narrow detection ranges and broad peaks: only a few types of biomarkers are detected at one time because screening a sample for multiple biomarkers takes a long time, a lot of agents and effort. Magnetic properties of nanoparticles cannot be

altered due to a lack of particle-specific properties [22], which means that one magnetic nanoparticle cannot be distinguished from others based on its magnetic properties. Similarly, electric measurements cannot distinguish two types of metallic or semiconductive nanoparticle, as electric signals are dependent on the arrangement of nano particles in aggregate [23]. Although few types of nanoparticles can be distinguished from their redox potentials in electro-chemical analysis, the detection range only spans a few volts and peaks are too broad to offer high multiplicity [24]. For any spectroscopic methods, the gap between two adjacent peaks should be sufficiently large to differentiate two peaks from each other (**Figure 1A**) [25]. The optical absorbance peak of semiconductor or metal nanoparticles is broad with full width at half height of 150 nm, which limits the multiplicity of detection in the wavelength range of 400–900 nm to several due to peak overlapping [26]. In cases of surface-enhanced Raman spectroscopy, where sharp peaks exist over a broad spectral range [27], available Raman dyes are limited, the ratio of Raman active nanoparticles is low and the reliable signals for quantitative analysis are difficult to obtain. Rare-earth up-converting nanobarcodes have been generated for multiplexed biological detection, but the method needs to use a fluorescence microscope to image nanobarcodes attached to microspheres [28], which is time-consuming. Semiconductive nanowire array or nanomechanical cantilever array can be used to detect several biomarkers at the same time [29,30], but they need localized surface patterning or modification, and the complexity of the readout system will increase exponentially with multiplicity. In addition, if biomarkers can be detected in body fluids, sample preparation will be simplified and the loss of biomarkers will be reduced, but most optical detections require extensive efforts to prepare samples free of colored molecules; otherwise the error associated with the readout will compromise detection sensitivity.

Solid-to-liquid phase-change

During the melting process, solid materials absorb heat without a rise in temperature according to Gibbs' phase rule. **Figure 1B** shows the temperature change of a solid as a function of adsorbed heat, in which the first flat region corresponds to the solid-to-liquid phase-change [31]. As long as a solid phase exists, all absorbed heat is used to melt the solid and the temperature of the solid–liquid mixture is constant. The fusion enthalpy (ΔH) is proportional to the latent heat of fusion and the mass of material. Although the second flat region corresponding to the liquid–vapor phase-change has a larger enthalpy change, the large volume change makes it difficult to use [32]. The fusion enthalpy and melting temperature can be determined by using differential scanning calorimetry (DSC), where the heat flux of a sample relative to the reference is measured during a linear thermal scan, the melting peak will be sharp, with peak width dependent on the size and thermal conductivity of the material, ramp rate and instrument. All materials exhibit solid–liquid phase-change at certain temperatures: metal has a relatively large volumetric latent heat of fusion when compared with paraffin; the fusion enthalpy of metal or alloy depends on its mass or composition, and its latent heat of fusion; the melting point depends on the atomic number (metal) or composition (alloy), providing that the size of material is larger than the critical size (20 nm), below which surface atoms play an important role in reducing the melting point [33,34]. A panel of metallic alloys that have composition-dependent melting points

between 100 and 700°C can be designed based on phase diagram knowledge gained previously, and prepared to form nanoparticles using colloid method. Eutectic alloys go directly from solid to liquid states without pasty stage and have sharp melting peaks similar to metals. Although miscible solids could be formed when metals with the same structures are mixed (e.g., gold and silver), most alloys have eutectic compositions.

Thermal biosensing with phase-change nanoparticles

Instead of relying on existing optical, electric, magnetic and electrochemical approaches for signal transduction, we have developed a new biosensing method (i.e., thermal biosensing) in which nanoparticles of solid–liquid phase-change materials are used as thermal probes to detect multiple biomarkers (proteins or oligonucleotides) (**Figure 2**). The nanoparticles will first be modified with ligands that can bind with biomarkers, and then incubated in buffer or body fluid, which contain biomarkers. After incubation, a high thermal conductivity solid substrate (silicon or aluminum), modified with multiple ligands (different from the ones on the nanoparticles), will be immersed into the solution to capture nanoparticles by forming sandwiched complexes. After washing away unbound nanoparticles, those attached onto the substrate will be read out using DSC, where the heat fluxes of the sample and a reference are measured simultaneously during a linear thermal scan. If there is a phase-change of materials, extra heating power has to be input into the sample in order to minimize the temperature difference compared with the reference. The added heat can be plotted as a function of temperature to determine the enthalpy of fusion and melting temperature, from which the nature and amount of the biomarker can be determined. A series of alloy nanoparticles with composition-encoded melting points can be used to detect multiple protein and DNA biomarkers after creating a one-to-one accordance between one type of nanoparticle and one type of biomarker. When metal particles are sufficiently small (diameter less than 20 nm), their melting temperature is dependent on their diameter (**Figure 3A**) [35]. Similar trends have been found in other nanoparticles [36]. However, the dependence of the melting point of nanoparticles on size shows an exponential trend, which means that in order to make nanoparticles with a certain melting temperature, the size of the nanoparticles will have to be precisely controlled, which is a great challenge for synthesis. For nanoparticles with a diameter larger than the critical size (20 nm), the melting temperatures are close to their bulk melting temperature.

High multiplicity

By using normal DSC equipment, the peak widths at half height of metal nanoparticles and eutectic alloy nanoparticles can be less than 1°C (0.6°C) at a ramp rate of 1°C/min. If the thermal scan range is from 100 to 700°C, the maximal number of melting peaks that can be resolved will be 1000 according to Rayleigh's criterion on spectral resolution, which means that over 1000 different types of biomarkers can be simultaneously detected in one thermal scan by detecting their associated nanoparticles. **Figure 3B** shows the relationships between ramp rates and melting peak widths of nanoparticles, where the peak width is less than 1°C at a ramp rate of 1°C/min. According to Gray's model, the melting peak can be treated as two half-peaks: the first is a straight line and the second is an exponentially decaying curve that stops when the heat flow rate is 1% of its maximum. The peak width can be derived

from thermal resistance (R), specific heat (Cs), ramp rate (β) and fusion enthalpy (ΔH) using [37]:

$$w = RC_s \left[\sqrt{1 + \frac{2\Delta H}{RC_s^2 \cdot \beta}} - 1 + \ln 100 \right] \cdot \beta$$

Composition-encoded nanoparticles

Although metal nanoparticles have sharp melting peaks, only a small number of metals in the periodic table can be used to produce nanoparticles due to availability and safety issues. Alloy nanoparticles with eutectic compositions have single sharp melting peaks, and the melting points are determined by compositions (**Figure 3C**) (providing that their sizes are larger than the critical size) [38]. According to the combination rule, if any two out of three metals can form binary eutectic alloys, the three metals can form one ternary eutectic alloy and three binary eutectic alloys; and the total number of metals and alloys will be seven. In general, for a given number of metals that form binary eutectic alloys, among any two of them, the numbers of binary alloys, ternary alloys and so on can be derived graphically from Pascal's triangle, and the total number of metals and eutectic alloys can be derived from:

$$\sum_{k=1}^n C_n^k - 1 = \sum_{k=1}^n \frac{n!}{k!(n-k)!} - 1 = 2^n - 1$$

where n is the total number of metals and k is the number of metals inside one nanoparticle. We have identified ten different metals that can form binary eutectic alloys among any two of them. These metals include aluminum, bismuth, cadmium, copper, gallium, indium, lead, magnesium, palladium and silver. These metals can be used to form at least ten types of metal nanoparticles, 45 types of binary alloy nanoparticles, 120 types of ternary eutectic alloy nanoparticles, 210 types of quaternary eutectic alloy nanoparticles and so on. The total number of metals and eutectic alloys that can be formed is 1023. Note that the combination corresponding to no metals (n = 0) is removed. Nanoparticles of these metals and eutectic alloys have sharp and discrete melting peaks that can be resolved by DSC with high peak resolution (0.01°C). In order to determine the compositions of eutectic alloys, Computer Coupling of Phase Diagrams and Thermochemistry software is used [39], where the parameters used to derive total free energies can be derived by fitting available binary phase diagrams. **Figure 3D** shows the melting temperatures and latent heats of fusion of 50 types of eutectic alloys, which are selected in such a way that the melting peaks are evenly distributed over the whole temperature range. A group of ten metals and alloys has been identified and synthesized (**Table 1**) that can be used for biosensing.

Theoretical detection limit

Taking the root mean square noise of a commercial DSC instrument (DSC 7; Perkin Elmer, MA, USA) as 0.2 μ W, the minimal detectable heat flow will be 0.2 μ J for a 1°C wide peak at a ramp rate of 1°C/sec. If 30-nm diameter copper nanoparticles with latent heat of 205 J/g and density of 13.6 g/cm³ were used, the number of nanoparticles that can absorb 0.2 μ J heat

during phase-change will be 2.6×10^6 . Providing that one antibody is attached on each nano particle, the correlating antigen concentration in a 1 ml solution will be 4.3×10^{-15} M or 4.3 fM. By using large nanoparticles or materials with high latent heat, the detection sensitivity can be increased. It should be noted that the calculation is only for the best case scenario for DSC equipment with particular sensitivity. In practice, it is not the case that one antibody binds to one nano particle owing to diffusion, affinity and limitations. However, people can always use DSC with higher sensitivity to achieve a better detection limit.

Nanoparticle synthesis

Nanoparticles of metals and eutectic alloy, such as indium, tin, bismuth and lead–tin eutectic alloy, can be produced by thermally decomposing organometallic precursors at stoichiometric ratios in the presence of surfactants (**Figure 4**). By changing molar ratios of the surfactant and precursor, nanoparticles with diameters between 30 and 200 nm can be made. **Figure 4** shows a transmission electron microscopy image of bismuth nanoparticles. DSC measurement confirms that the melting point of bismuth nanoparticles is the same as bulk bismuth (271°C) and the peak area is proportional to the mass (**Figure 4C**).

Uniqueness

The unique features of thermal biosensing using phase-change nanoparticles include: high multiplicity owing to a sharp melting peak, large scan range and wide choice of materials; high sensitivity and an adjustable detection range using nanoparticles of various sizes, composition and ligand grafting density; minimal sample preparation by detecting biomarkers contained in body fluid; and simultaneous detection of different biomarkers (i.e., proteins and oligonucleotides) at the same time, under the same conditions. Although an enzyme thermistor can be used to detect heat absorption and the evolution of the biochemical reaction associated with analytes, the multiplicity of thermistor detection is low and only one species can be detected at one time. Nanoparticles of low melting temperature metals and alloys have been made as solder materials or heat transfer additives, but there is no attempt to use them for biomarker detection due to a lack of awareness of their unique thermal properties for multiplexed detection of molecular biomarkers.

Application examples of thermal biosensing

Multiplexed protein detection

Thermal biosensing has been used to detect proteins with nanoparticles of indium and a lead–tin alloy [40]. The nanoparticles are generated by the colloid method and modified to have amine groups, together with an aluminum substrate with native oxide. The nanoparticles and aluminum surface are conjugated with biotin by incubating with an amine-reactive biotinylation reagent, followed by washing with dimethyl sulfoxide and phosphate-buffered saline (PBS). **Figure 5A** shows DSC curves of indium nanoparticles attached on the aluminum surfaces at different avidin concentrations at a thermal ramp rate of 10°C/min, where the heat flux increases as avidin concentration increases. The melting peaks at 156°C confirm that indium nano particles have been immobilized on the aluminum surface. Similarly, lead–tin alloy nanoparticles have been used to detect rabbit IgG, where heat flow is proportional to the concentration of rabbit IgG.

The multiplicity of thermal detection is reflected in simultaneous detection of four proteins (rabbit IgG, human IgG, biotin and PSA) using nanoparticles of indium, lead–tin, tin and bismuth nanoparticles that are modified with the associated antibodies. In order to modify aluminum surfaces, secondary antibodies are mixed at the same molar ratio and immobilized on amine-modified aluminum substrate. Multiplexed detection is performed by incubating modified aluminum substrate in a mixture of 2 ng/ml of each protein in PBS (pH 7.4). After washing, the surface is incubated in a mixture of four types of surface-modified nanoparticles, followed by a second washing. The aluminum substrate is tested by DSC. **Figure 5B** shows four melting peaks (156, 183, 230 and 270°C) that correspond to four types of nanoparticles. The difference in heat flows of the four peaks is induced by differences in enthalpy of fusion of nanoparticles and the grafting density of antibodies on nanoparticles [41].

Multiplexed DNA detection

Thermal detection does not rely on optical transparency and is immune to inference from colored species. This method has been used to detect single stranded oligonucleotides (ssDNAs) in body fluid [42]. Target ssDNAs are added to milk or blood to make samples that are not transparent. The probe ssDNA-modified nanoparticles and capture ssDNA-modified aluminum surface are added into solution for hybridization. After hybridizing for 3 h, the aluminum surface is taken out from the solution, washed with buffer and tested by DSC. DSC curves show that lead–tin nanoparticles are collected at different concentration of target ssDNA. The method has been used to detect two types of ssDNA by modifying capture and probe ssDNA on the aluminum surface, and two types of nano particles (i.e., indium and lead–tin alloy). Meanwhile, two target ssDNAs are dispersed in PBS at pH 7.5 and 1-M NaCl. The probe ssDNA-modified indium and lead–tin nanoparticles, as well as the capture ssDNA-modified aluminum surfaces are immersed into PBS that contains two types of target ssDNAs. After hybridization, the aluminum surface is washed with a PBS. DSC results show the melting peaks of indium and alloy nanoparticles, thus confirming the existence of two types of target ssDNA in the solution.

Enhance detection sensitivity with nanostructured substrate

The sensitivity of thermal biosensing depends on the mass and the latent heat of fusion of immobilized phase-change nanoparticles. Increasing the surface area of substrate can enhance the sensitivity of detection [43]. Silicon substrate with vertically aligned high-aspect-ratio nanopillars are made by combining nanosphere lithography and gold nanoparticle-enhanced etching of silicon. **Figure 5C** is a scanning electron microscope image of a nanopillar (200 nm in diameter and 2 µm in height). The surface area of the substrate with nanopillars is ten-times larger than that of a flat surface of the same size. The nanopillar surfaces have been modified by 3-aminopropyl-triethoxy silane and activated by disuccinimidylsuberate, followed by conjugating with an aptamer specific to thrombin. The sequence of the aptamer is 5'-AmMC6-TTTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3'. The substrate is immersed into a buffer containing different concentrations of thrombin. After washing, the substrate is immersed in another buffer that contains nanoparticles modified with a different aptamer with a sequence of 5'-TTTTTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3'. The normalized sensitivity (peak area

per mm²) on the nanopillar silicon surface is enhanced by four times at a concentration of 55 nM compared with flat aluminum surfaces.

Detecting multiple biomarkers with large concentration differences

The concentration detection range can be estimated using the two parameters discussed here. DSC allows the ramp rate to be adjusted in the range of 0.02–500°C/min, meaning that the detectable heat flow (concentration range) can be adjusted over four orders of magnitude. The diameter of nanoparticles can be changed from 30 to 200 nm. The grafting sites on nanoparticles can be any number from one to $4\pi \times 1000$. The latent heat of fusion can be changed from 23 J/g for indium to 205 J/g for copper. The upper limit of concentration will be 2.5×10^{14} and the lower limit will be 1.6×10^3 ; therefore, the concentration range is derived as 11 orders of magnitude. This method has been used to detect two DNA biomarkers at concentrations of 100 nM and 100 pM, spanning three orders of magnitude, by controlling grafting density of the ligand on the nanoparticle, using different nanoparticles and performing thermal analysis at different ramp rates [44].

Silica-encapsulated phase-change nanoparticles as thermal barcodes

The composition-dependent melting point and sharp melting peak of nanoparticles allow for creation of huge number of microspheres that have individually distinguishable signatures.

Table 1 shows that from ten types of metals, it is possible to form 1023 types of nanoparticles that have distinguishable melting peaks. If 100 types of nanoparticles can be selected from a group of 1023 types of nanoparticles, it is possible to form thermal barcodes that have a labeling capacity of over billions. A panel of phase-change nanoparticles, including indium, lead–tin alloy, tin and bismuth, is selected and embedded in silica microspheres using the colloid method (**Figure 6A**) [45]. The melting enthalpies (peak areas) are proportional to mass and latent heat of fusion of nanoparticles. The number of thermal probes formed by four types of nanoparticles is 15. **Figure 6B** shows DSC curves collected from 16 different types of silica microspheres. Each melting peak can be denoted as 1 or 0 depending on whether there is detectable heat flux or not. The 16 combinations of four elements are 0000, 1000, 0100, 0010, 0001, 1100, 1010, 1001, 0110, 0101, 0011, 1110, 1101, 1011, 0111 and 1111. The height and area of each peak corresponds to the amount of certain nanoparticles in microspheres and each barcode can be decoded by counting melting peaks in a DSC curve.

Conclusion & future perspective

As more molecular biomarkers are discovered for most diseases through high-throughput techniques, the need for multiplexed detection of biomarkers becomes more intensive. It was proven that the use of multiple biomarkers could greatly enhance the detection reliability for a variety of diseases, such as cancers, cardiovascular disease, Alzheimer's disease and autism [46]. The multiplexed detection can be used to monitor disease progression and find characteristic changes in multiple biomarkers. In addition, multiple biomarkers can also be used to classify patients into different subgroups and find the most effective treatment for individual patients. The multiple biomarkers of a variety of infectious diseases can be tested

simultaneously to rapidly identify the type of infectious disease to ensure effective treatment. This technique can also be used to detect multiple biological weapons.

The thermal biosensing is based on nanoparticles of solid-to-liquid phase-change material, where each type of nanoparticle is conjugated with ligands of according protein and DNA biomarkers, and immobilized on ligandmodified substrates by forming a double-helix DNA chain or sandwiched antibody–antigen complex. The type and concentration of biomarkers are reflected in the melting temperature and fusion enthalpy of nanoparticles in DSC. Owing to sharp melting peak and a large thermal scan range, thermal biosensing allows detection of multiple biomarkers at the same time. However, there is still research that needs to be performed before thermal biosensing can be widely adopted by research and industrial communities.

Surface modification

The use of multiple metallic species makes surface modification complicated. The surface chemistry needs to be fine tuned for different species. Another technique is to use a universal coating, such as silica, around each type of nanoparticle to utilize the general protocols to modify the silica surface [47–48].

Relatively slow thermal readout & bulk instrument

The thermal readout is a linear temperature rise process, where the normal thermal ramp rate is 10–50°C/min. The normal DSC instrument is too bulky for portable diagnosis. Although sufficient for research application, it is considered inadequate for field deployable application, where rapid detection is crucial. DSC can be miniaturized as a portable device [49], and can be made to have an extremely high ramp rate (2.4 million °C/min in the case of flash analysis) due to the small thermal masses of the thin-film heating and temperature measuring elements [50].

Grafting density of the ligand

The sensitivity of thermal biosensing depends on the grafting density of the ligand on the nanoparticle. To date, the grafting density can only be controlled by diluting ligands. Ideally, a precise number of ligands should be used in order to quantitatively determine the concentration of biomarkers. A possible way to control grafting density is to use protein nanocages derived from a virus to encapsulate each nanoparticle, where each protein nanocage has a precisely controlled binding site. However, not much work has been performed in this area.

Specificity or selectivity of detection is determined by biological interactions such as those between proteins & DNAs

An issue of multiplexed detection is the possible crossreactivity of the antigen–antibody interaction, but, in most cases, crossreactivity can be avoided or minimized by selecting antigen–antibody pairs that have minimal cross-interaction. An important direction for DNA detection is to test whether this method can be used to detect single-nucleotide mismatch.

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Executive summary

- The thermophysical properties of phase-change nanoparticles (sharp melting peak, composition-dependent melting temperature and large latent heat of fusion) allow highly sensitive detection of multiple biomarkers for early detection of diseases.
- Eutectic alloy nanoparticles can be designed and made to have controlled melting temperatures distributed over a large temperature range.
- The detection range of thermal biosensing can be adjusted over several orders of magnitude by changing nanoparticle size, materials, grafting density and thermal scan rate.
- Thermal biosensing with phase-change nanoparticles can be used to detect multiple protein and DNA biomarkers at the same time in the same sample with minimal sample preparation.
- A panel of phase-change nanoparticles can be encapsulated inside non-melting shells to form thermal barcodes that have an extremely high labeling capacity.

Figure 1. Peak overlap and temperature-heat relationship in phase change

(A) Spectral resolution of any spectroscopic method, where two adjacent peaks have to be separated at a certain distance in order to be differentiated from each other. (B) The temperature of a material as a function of added heat, where the first flat region corresponds to the solid-liquid phase-change, suggesting that the temperature of the material does not rise until all solid are molten.

ΔH_{fus} : Fusion enthalpy; ΔH_{vap} : Vaporization enthalpy; ΔT : Temperature change; c : Specific heat; m : Mass.

Figure 2. Thermal biosensing

Nanoparticles modified with ligands are incubated in a solution containing biomarkers, immobilized on a substrate modified with multiple ligands and then a readout is produced using differential scanning calorimetry (inset), where the peak position and area indicate the nature and amount of biomarker, respectively.

DSC: Differential scanning calorimetry.

Figure 3. Designing metals and eutectic alloys

(A) Melting temperature as a function of particle size; the figure labels are modified for observation purpose. (B) Relationship between peak width at half height of the melting peak and thermal ramp rate. (C) A binary phase diagram, where black dashed lines are liquidus lines. (D) Latent heat of 50 eutectic alloys whose melting temperatures are evenly distributed between 100 and 650°C.

Figure 4. Nanoparticle synthesis and characterization

(A) Production of alloy nanoparticles using thermal decomposition. Transmission electron microscopy image of (B) large and (C) small bismuth nanoparticles. (D) A differential scanning calorimetry curve shows the melting point of bismuth nanoparticles is the same as bulk bismuth (271°C) and the peak area is proportional to the mass.

Figure 5. Thermal biosensing with phase-change nanoparticles

(A) Differential scanning calorimetry curves of indium nanoparticles immobilized on aluminum surface through biotin–avidin interactions, where the curves from up to down are at avidin concentrations of 20, 2 and 0.05 ng/ml, respectively. (B) Differential scanning calorimetry curve of four types of nanoparticles after detect four different types of proteins. (C) Silicon nanopillars made by metal nanoparticle-assisted etching.

Figure 6. Thermal barcodes enabled with phase-change nanoparticles

(A) Thermal barcode formed by encapsulating a panel of phase-change nanoparticles inside a silica shell. (B) A panel of differential scanning calorimetry curves were collected from a series of silica microspheres that contain different ratios of nanoparticles of lead–tin, tin and bismuth.

Table 1

Ten types of metals and alloys and their melting temperatures.

Metal	Composition (% mass)	Melting point (°C)
Field's metal	32.5 bismuth; 16.5 tin; 51 indium	58
Indium–bismuth eutectic alloy	66.3 indium; 33.7 bismuth	74
Fusible alloy 203	52.5 bismuth; 32 lead; 15.5 tin	95
Indium–tin eutectic alloy	52 indium; 48 tin	
Fusible alloy 255	55.5 bismuth; 44.5 lead	124
Fusible alloy 281	58 bismuth; 42 tin	138
Indium	100 indium	157
Tin63lead37 alloy wire	63 tin; 37 lead	183
Bismuth	100 bismuth	271.5
Zinc	100 zinc	419.5